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Changes of Human Glomerular Basement Membrane in Diabetes Mellitus

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Summary: Glomerular basement membranes were isolated from kidneys of human diabetics and non-diabetics. Albumin and immunoglobulin G content of glomerular basement membranes as determined after protease digest was higher in the diabetic group while lower values were obtained for heparan sulphate. Nonenzymatic glucosylation of whole glomerular basement membranes and tendon tissue was significantly elevated in diabetic subjects. Correlation of the determined parameters suggest that charge and size selective properties are altered in diabetic glomeruli. Serum albumin and albumin deposited in glomerular basement membranes showed the same content of nonenzymatically bound glucose in a normal and in a diabetic subject, respectively. Thus it would appear that in human diabetes the glucosylated albumin does not underly increased transcapillary transport as was reported in vitro.

Änderungen der menschlichen glomerulären Basalmembran bei Diabetes mellitus

Zusammenfassung: Glomeruläre Basalmembranen wurden aus Nieren von Diabetikern und Nichtdiabetikern isoliert. Der nach Pronaseverdauung bestimmte Albumin- und Immunglobulingehalt der glomerulären Basalmembranen war beim diabetischen Kollektiv höher als beim Vergleichskollektiv, während der Heparansulfatgehalt des diabetischen Kollektivs niedriger war als im Vergleichskollektiv. Sowohl glomeruläre Basalmembranen als auch Sehnengewebe waren bei Diabetikern signifikant höher nicht-enzymatisch glucosyliert. Die Korrelationen der gemessenen Kenngrößen legen die Vermutung nahe, daß bei diabetischen Glomeruli sowohl die Ladungs- als auch die Poreneigenschaften verändert sind. Sowohl bei Diabetikern wie Nichtdiabetikern war das Serumalbumin und das aus glomerulären Basalmembranen isolierte Albumin gleich hoch glucosyliert. Dieses Ergebnis zeigt, anders als in vitro Studien, daß glucosyliertes Albumin von Diabetikern nicht schneller transkapillar transportiert wird als das der Kontrollgruppe.

Introduction

Microvascular lesions constitute a cardinal feature of diabetic late complications. Although it has been recognized for some time that ultrastructural and biochemical alterations of the renal glomerular basement membrane coincide with diabetic nephropathy, this relationship is not fully understood. Biochemical analysis of glomerular basement membrane from normal and diabetic subjects revealed alterations in neutral sugar (1) and heparan sulphate content (2). Further, immunofluorescence studies of diabetic kidneys have demonstrated intense linear staining for human albumin and immunoglobulin G in glomerular basement membrane and *Bowman*'s capsule (3, 4) consistent with clinical findings of an accelerated transcapillary escape of serum proteins in diabetes (5). Changes in the composition of basement membranes are likely to be responsible for functional disturbances and hence for the development of capillary disease. We have therefore quantified the amount of nonenzymatically bound glucose, of heparan sulphate, and of albumin and immunoglobulin G of glomerular basement membrane prepared from kidney obtained at autopsy from normal and diabetic humans.

Materials and Methods

Kidneys from six subjects with longstanding (6–20 years, mean 13.2) maturity-onset diabetes and from ten normal subjects were obtained at autopsy. The mean age (range) for diabetics and non-diabetics was 63.8 (46–83) years and 66.2 (40–86) years, respectively. Data on diagnosis, onset, duration, therapy and on diabetic late complications were taken from the case histories. Nephropathy was diagnosed at autopsy by macroscopic and microscopic examination. The kidneys were maintained at −20 °C until thawed for isolation and preparation of glomerular basement membrane.

Glomeruli were isolated from the cortical portion of kidneys by the sieving procedure through stainless-steel screens according to *Krakower* et al. (6). Isolation of glomeruli and of glomerular basement membrane was monitored by phase-contrast microscopy. Glomerular basement membranes were obtained by sonification and detergent treatment as described by *Meezan* et al. (7).

Precisely weighted portions of pure glomerular basement membrane (10–30 mg) were treated with highly purified collagenase (Sigma St. Louis Mo. USA) as described by *Parthasarathy* et al. (8). After three days the samples were centrifuged and the supernatants removed for determination of albumin and immunoglobulin G. The residue was resuspended in 0.1 mol/l Tris-acetate pH = 8.0 and further digested with pronase as described in l.c. (8). After three days traces of insoluble material were removed by centrifugation and the heparan sulphate-containing supernatant was chromatographed on DEAE-cellulose (8). Heparan sulphate-containing fractions eluted with 3 mol/l pyridine formate were lyophilised. The uronic acid content was determined by a micromodification of the method of *Blumenkrantz* et al. (9) using glucuronic acid as standard. Purity and chemical nature of glycosaminoglycan was assessed by electrophoresis and nitric acid treatment according to l.c. (8).

Albumin and immunoglobulin G were determined immunologically with the Immunochemistry Analyzer (Beckman Inc. Brea USA). 5, 10 or 20 mg/l albumin or immunoglobulin G were dissolved in the collagenase digestion buffer as standards. The assay was linear from 5–50 mg/l and duplicates did not differ by more than 15%. For purification of albumin trapped in the glomerular basement membrane, collagenase digest supernatants from normal and diabetic basement membranes were pooled, dialysed against water and lyophilised. The residue was dissolved in 600 µl H_2O and purified by gel filtration. The high performance liquid chromatograph consisted of a solvent delivery system Model 6000 A, an automatic injector, Model WISP 710 A and an UV-absorbance detector, Model 440 (all from Waters Ass. Milford MA 01757). The column G 4000 SW (60 cm × 0.75 cm) was obtained from LKB (Munich, GFR). Phosphate buffered saline was used as eluent and the fractions were monitored at 280 and 254 nm with absorbance unit full scale = 0.05. The column was calibrated with human serum albumin (Behring, Marburg, GFR). Fractions (1.5 ml) were collected and tested for albumin content as described above. Albumin eluted as a single peak well separated from other proteins. The purity of the albumin fractions was assessed by immunoassay/A_{280} nm ratios. The fraction with high albumin content (at least 85% pure) was lyophilised, hydrolysed, and analysed for nonenzymatically bound glucose as described previously (10). Nonenzymatically bound glucose of glomerular basement membrane or tendon tissue was determined according to l.c. (11).

Results

The amount of nonenzymatically bound glucose of tendon and glomerular basement membrane from diabetic and nondiabetic individuals is presented in figure 1. On the average, the levels in diabetics are at least two times higher than in non-diabetics, the difference being significant at $P < 0.005$ for tendon and glomerular basement membrane. None of the values of the diabetic group lies within the normal range.

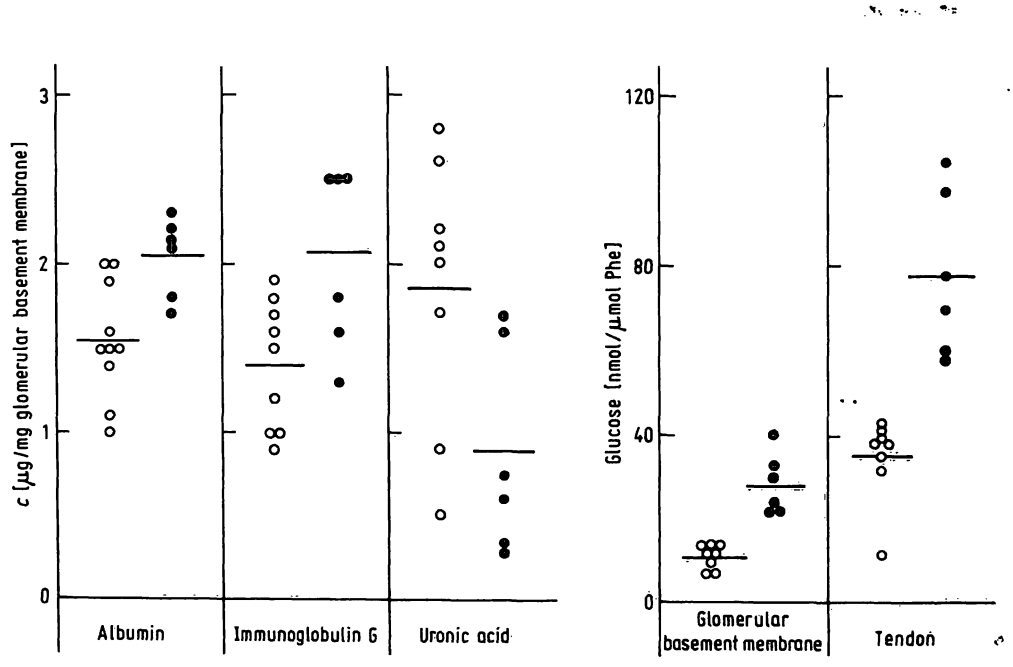

Fig. 1. Albumin, immunoglobulin G and uronic acid content of human glomerular basement membrane from kidney of normal (○) and diabetic (●) subjects.
Nonenzymatic glucosylation of tendon and glomerular basement membrane of the same subjects was also determined and related to phenylalamine content.

These results are in agreement with previous observations (11). Figure 1 further illustrates that glomerular basement membrane of diabetic kidneys also contained higher amounts of albumin and immunoglobulin G as compared to non-diabetics. The differences were significant at a level of $P < 0.05$. *Michael* and *Brown* (12) using a different method found a mean albumin content of 1.31 and 2.25 μg/mg glomerular basement membrane for normal and diabetic glomerular basement membrane, respectively, in good agreement with our results (1.55 and 2.05). That our values for diabetic glomerular basement membrane are somewhat lower is probably due to the fact that we studied an unselected diabetic group whereas *Michael* and *Brown* (12) were dealing with surgically removed kidneys from diabetic patients suffering from terminal nephropathy and destined for renal transplantation.

That albumin was not merely adsorbed to glomerular basement membrane during preparation but firmly bound (probably trapped in the glomerular basement membrane lattice) was indicated from the time course of its release during collagenase incubation. After one hour no albumin could be detected. After one day 40–60% of the albumin was liberated, and after two days 80–100% was present in the supernatant with little or no change after three days of incubation.

From our data in figure 1 it appears that there is also more immunoglobulin G present in glomerular basement membrane of diabetic subjects (mean 2.03 μg/mg) than in glomerular basement membrane of normal individuals (mean 1.40 μg/mg) although the difference is significant only at $P < 0.05$. To our knowledge no quantitative data on the immunoglobulin G content of human glomerular basement membrane exist so far in the literature. From immunofluorescence studies there is however evidence that in diabetic patients immunglobulin G passes the capillary easier than in normals (5).

Confirming recent findings by l.c. (2) figure 1 further indicates that glomerular basement membrane from diabetic subjects contains less heparan sulfate (determined as hexuronic acid) as compared to glomerular basement membrane obtained from normal individuals.

Although the uronic acid content covers a large range the difference is significant at the $P < 0.01$ level. Pertaining to the development of diabetic microangiopathy it has been reported that albumin with a higher ("diabetic") level of nonenzymatically bound glucose can pass the capillary membranes much faster than normal albumin, in vitro (13). Accordingly one should expect an enrichment of the higher glucosylated albumin species in extracellular deposits. Using a sensitive and specific method (10) we were able to quantify nonenzymatically bound glucose in 30–50 μg of albumin isolated from glomerular basement membranes. The content of lysine-bound glucose of the albumin from glomerular basement membrane of normals and diabetics was 3.8 and 10.4 nmol/mg protein respectively; these values compare well with the levels of glycosylated albumin isolated from the serum of normals (mean 3.6 ± 0.3, n ± 52) and diabetics (mean 10.8 ± 3.3, n = 52). These results are not consistent with a preferential extravasation and deposition of (higher) glucosylated albumin, at least in the case of glomerular basement membranes.

Discussion

The present study confirms previous results that human renal diabetic basement membranes contain a higher amount of albumin (5) and a lower amount of heparan sulphate (2) than do membranes derived from normal kidney. Furthermore we detected higher amounts of immunoglobulin G in glomerular basement membrane of diabetics as compared to normals. When immunoglobulin G and albumin contents of glomerular basement membrane were correlated (fig. 2) a reasonable, positive correlation was obtained. If, as currently believed (14–16), the per-

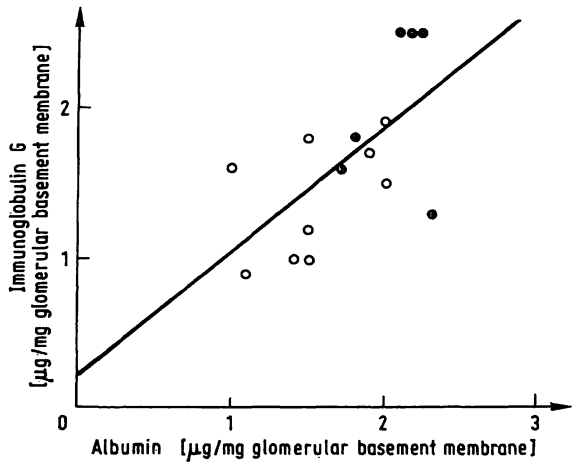

Fig. 2. Comparison of albumin and immunoglobulin G content in glomerular basement membrane from kidney of normal (○) and diabetic (●) subjects. y = 0.83x + 0.20; r = 0.619.
The serum proteins were released from glomerular basement membrane after digestion with collagenase as described under methods.

meability of glomerular basement membrane is defined by pore size and anionic sites, a knowledge of the number of included proteins and their charge and size differences should permit predictions as to the possible changes in filtration properties. Depletion of negative charge of the basal membrane is likely to enhance transglomerular passage of circulating negatively charged proteins like albumin (isoelectric point 4.7), whereas this is not expected to apply for immunoglobulins G which are essentially neutral in plasma (isoelectric point 6.6–8.0). In agreement with this view is the observation that proteinuria of diabetics is of high selectivity (17, 18). Our results on the composition of glomerular basement membrane may be interpreted as a combination of defects in the diabetic state – i.e. a decrease in negative charge and an increase in pore size. Significantly decreased heparan sulphate per amino acid content (8) or per mg glomerular basement membrane (this study) was shown for the diabetic kidney. However, as the negative charge is localised at the laminae rarae externa and interna (19) it is possible that the reduction in heparan sulphate in diabetes is simply a matter of calculation relative to a greater mass of collagen (basement membrane-thickening). On the other hand, reduced $^{35}SO_4^{2-}$ incorporation in glomerular basement membrane, indicating reduced heparan sulphate synthesis, has been observed in diabetic rats (20, 21). When albumin or immunoglobin G content and uronic acid content of glomerular basement membrane were compared (tab. 1) a weak negative correlation was found (−0.40 and −0.63). Thus defective sieving with respect to molecular size is likely to occur in diabetic glomeruli, and a reduced negative charge may also influence the permeability properties. *Myers* et al. (22), studying extensively the glomerular barrier function in normal and diabetic subjects, found increased urinary immunoglobulin G excretion when urinary albumin concentration was increased. They concluded that the development of large pores within the glomerular membrane in advanced diabetic nephropathy permits passage of large plasma proteins into the urine.

Cohen et al. (23) suggested that nonenzymatic glucosylation of lysine ε-amino-groups of glomerular basement membrane interferes with crosslinking in the collagen framework thus leading to the development of larger pores. As indicated in table 1 we have found only very weak correlation between nonenzymatic glucosylation of glomerular basement membrane and albumin or immunoglobulin G. Glomerular basement membrane from diabetic kidneys with the highest immunoglobulin G content showed the lowest nonenzymatic glucosylation, which may be explained by a shorter half life of glomerular basement membrane collagen (repair mechanism). Taken together, our results do not support the view that nonenzymatic glucosylation of glomerular basement membrane per se changes filtration properties.

As nonenzymatic glucosylation of tendon tissue reflects probably the achievement of metabolic control in diabetics over a long period of time (11), these values were also correlated with the albumin and immunoglobulin G contents of glomerular basement membrane. A rather good positive correlation was found (tab. 1) confirming former results that long term bad metabolic control of diabetic patients leads to proteinuria, especially albuminuria. Our findings suggest that we are not dealing with enhanced nonenzymatic glucosylation of glomerular basement membrane leading to diabetic proteinuria, but rather with "dysregulation" leading to synthesis of defective basement membrane.

Tab. 1. Linear correlation of albumin, immunoglobulin G and uronic acid content of glomerular basement membrane from nondiabetic and diabetic individuals with nonenzymatic glucosylation of tendon and glomerular basement membrane and the albumin, immunoglobulin G and uronic acid content of glomerular basement membrane, respectively.

	Albumin	Uronic acid	Nonenzymatic glucosylation of glomerular basement membrane	tendon
Albumin	–	−0.40	0.50	0.65
Immunoglobulin G	0.62	−0.63	0.37	0.41
Uronic acid	–	–	−0.52	−0.19

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